deviations from single exponential relaxation, indicating two distinct phases. Of particular interest is the observation that the rapid phase has a rate that is 10-20 times faster than the bending rate observed in the IHF-H' complex. Thus, reducing the energetic cost of bending/kinking DNA speeds up the bending rate by nearly the same factor as the increase in binding affinity, indicating that the free energy of the transition state is lowered by the same amount as the free energy of the complex. These results support our earlier conclusion, that spontaneous bending of DNA is the first step in the recognition mechanism.

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Mechanisms of the Type I Restriction Enzyme EcoKI: Characterizing weak interactions using AFM

Kelly J. Neaves[1], David T.F. Dryden[2], J. Michael Edwardson[1], Robert Henderson[1].
[1]University of Cambridge, Cambridge, United Kingdom, [2]University of Edinburgh, Edinburgh, United Kingdom.

Common techniques for the characterization of biomolecular interactions are successful in detecting those of high affinity, but less effective at characterizing weak interactions. This limitation creates a methodological bias in investigations. Atomic force microscopy (AFM) provides a quick and gentle technique that allows the examination of biomolecules under near-physiological conditions. This means that direct observations of weak biomolecular interactions can be made, which in contrast with other techniques, prevents the necessity for averaging over a bulk number of molecules (typically $>10^9$).

Here we provide an example of how AFM can be used to characterize a biomolecular interaction, whose mechanism remains unclear after studies using other methods. The system studied is the DNA motor protein EcoKI. This is a bacterial type-I restriction enzyme which restricts the DNA of an invading virus. Restriction occurs between two sites and is preceded by the translocation of the intermediary DNA. The existing model for the enzyme was established over 30 years ago and involves two individual EcoKI monomers binding to two separate DNA sites. In this existing model the protein monomers would only meet after the DNA translocation. Using AFM we have shown that the enzyme monomers dimerize at one site, before any translocation, and that the dimerized complex then uses a diffusive looping mechanism to identify the secondary site. This demonstrates how AFM can be used to elucidate the mechanism of a well established macromolecular system. It also provides potential insight into the *in vivo* biology of type-I restriction-modification enzymes and other higher-order proteins. Such insights include: the kinetics and dynamics of site location; evolutionary implications; the protection of host DNA in restriction systems; space and volume considerations of large translocating complexes; and the positioning of sub-units in type-I systems.

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Transcriptional Activation by the Human Progesterone Receptor: Towards a Predictive Understanding

Keith D. Connaghan, Aaron F. Heneghan, Michael T. Miura, David L. Bain.
University of Colorado Denver Anschutz Medical Campus, Aurora, CO, USA.

A mechanistic and thus predictive understanding of transcriptional regulation in humans is highly lacking. For example, the current understanding for transcription factors such as progesterone receptor (PR) is that it binds to promoter regions of PR-regulated genes, then recruits coactivating proteins and RNA polymerase in order to activate transcription. However, this framework does not account for the ability of PR to differentially and simultaneously regulate multiple gene promoters. Differential regulation may hinge, at least in part, on the uniquely coded assembly of transcription factors at each promoter. In order to elucidate the mechanisms of promoter-specific binding, thermodynamic approaches were used to dissect PR interactions at natural and synthetic promoter sequences containing multiple binding sites. The results of experiments employing quantitative footprint titrations and statistical thermodynamic modeling show that PR-promoter interactions follow specific codes for assembly; that PR binding is highly cooperative; and that efficient coactivator recruitment is exclusively coupled to cooperative interactions. These results correlate with cellular measurements demonstrating that PR-regulated promoters containing multiple binding sites generate synergistic increases in transcriptional activity. Taken together, cooperativity may be key in the activation of transcription. As a means to assess the chemical forces responsible for cooperativity we examined the role of monovalent cations in regulating receptor-promoter interactions. Our findings suggest cooperative interactions are thermodynamically linked to Na^+ binding to PR. Noting that PR directly regulates the expression of ion channels and pumps, it is possible that Na^+ is not only an allosteric effector but also a physiologic regulator of PR-activated transcription in humans.

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Unravelling The Role Of Alba In The Organization Of The Archaeal Nucleoid

Maarten C. Noom[1], Felix J.H. Hol[1], Niels Laurens[1], Malcolm F. White[2], **Remus T. Dame**[1], Gijs J.L. Wuite[1].
[1]VU University, Amsterdam, Netherlands, [2]St Andrews University, St Andrews, United Kingdom.

Alba is one of the most abundant proteins in thermophilic and hyperthermophilic archaea and is believed to play an important role in DNA organization. It is a dimeric protein that binds DNA with no apparent sequence specificity. Earlier studies have shown that Alba is capable of bridging DNA duplexes, which may be key to its organizational role. However, a comprehensive understanding regarding the action of Alba in DNA organization is currently lacking. Using a combination of single-molecule imaging and micromanipulation techniques we now define mechanistic, structural and kinetic aspects of the Alba-DNA interaction. Thus, we demonstrate that Alba has two modes of action. Depending on its concentration and conformation, the protein either bridges two DNA duplexes or cooperatively binds to and stiffens a single DNA duplex. Based on these observations we put forward a structural model that describes the multi-modal behaviour of Alba in the context of the dynamic archaeal nucleoid.

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Regulation of the nucleic acid chaperone activity of HTLV-1 Nucleocapsid Protein

Ioulia Rouzina[1], Kristen Stewart-Maynard[1], Fei Wang[2], Margareta Cruceanu[2], Dominic Qualley[2], Dominic Qualley[2], Mithun Mitra[1], Robert Gorelick[3], Mark Williams[2], Karin Musier-Forsyth[4].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Northestern University, Boston, MA, USA, [3]AIDS Vaccine Program, Basic Research Program, SAIC-Frederick, Inc., Frederick, MD, USA, [4]Ohio State University, Columbus, OH, USA.

Nucleocapsid proteins (NC) of retroviruses are nucleic acid chaperones that facilitate nucleic acid remodeling. This property of NC proteins is critical for their role in viral genome dimerization, maturation and reverse transcription. In contrast to all other NC proteins studied to date, the human T-cell leukemia virus type 1 (HTLV-1) NC protein was shown to be an extremely poor chaperone. In this work, we demonstrate that the anionic C-terminal domain (CTD) of this protein is responsible for its poor chaperone function. Single molecule DNA stretching studies suggest that HTLV-1 NC dissociates very slowly from single-stranded DNA, which may be a primary reason for its poor chaperone activity. In contrast, a truncation mutant that lacks the CTD is a more effective annealing agent and displays faster off-rate kinetics. Under conditions of high ionic strength, the properties of the WT and CTD-deletion variant are much more similar to each other. Taken together, our data suggest that an electrostatic attraction between the anionic CTD and cationic N-terminal domain of HTLV-1 NC leads to polymerization onto ssDNA resulting in a poor ability to aggregate nucleic acids or to promote their annealing. This property of HTLV-1 NC makes it similar to typical SSB proteins, and may be related to this NC's role in excluding the viral restriction factor APOBEC3G from HTLV particles.

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DNA Interaction Properties of Nucleic Acid Chaperone Proteins from Retrotransposons

Kathy R. Chaurasiya[1], Fei Wang[1], Gaël Cristofari[2], Jean-Luc Darlix[2], Sandra L. Martin[3], Mark C. Williams[1].
[1]Northeastern University, Boston, MA, USA, [2]LaboRetro, Lyon, France, [3]University of Colorado School of Medicine, Aurora, CO, USA.

Nucleic acid chaperone activity is an essential component of reverse transcription in retroviruses and retrotransposons. Using DNA stretching with optical tweezers, we have developed a method for detailed characterization of nucleic acid chaperone proteins, which facilitate the rearrangement of nucleic acid secondary structure. The nucleic acid chaperone properties of the human immunodeficiency virus type-1 (HIV-1) nucleocapsid protein (NC) have been extensively studied, and duplex destabilization, nucleic acid aggregation, and rapid protein binding kinetics have been identified as major components of its activity. The chaperone properties of other nucleic acid chaperone proteins, such as those from the retrotransposons LINE-1 and Ty3, ORF1p and Ty3 NC, are not well understood. We used single molecule DNA stretching to characterize the activity of wild type and mutant ORF1p and Ty3 NC. ORF1p binds both double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) with high affinity, and strongly aggregates both forms. It is therefore an excellent chaperone, and altering certain residues has dramatic effects on chaperone activity. Wild type Ty3 also strongly aggregates both dsDNA and ssDNA, and melted DNA exhibits more rapid reannealing in the presence of Ty3 NC, relative to that observed in

the presence of ORF1p. We examine several Ty3 NC mutants to identify the roles of functional regions of the protein in its chaperone activity. This research was supported in part by funding from INSERM and ANRS (France).

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The Codes of "Non-Coding" ncRNA in Epigenetics: Episcription and Hermeneutics of the Genome in the Entangled Cancer-Angiogenesis-Tolerance Epigenome

Josef H. Wissler.
ARCONS Institute for Applied Research & Didactics, Postfach 1327, D-61231 Bad Nauheim, jhw@arcons-research.de, Germany.

OBJECTIVE: The genome as 3D-nucleic acid-[glyco-]protein nanomachine orchestrates translation of genetic [Mendelian] and epigenetic [non-Mendelian] information into phenotypical structural patterns. It is not restricted to transcription *and* translation of DNA scripts into proteins which matter only 2% of transcriptional output. By episcription, same genotypes [e.g. twins] deliver plethoras of not identical epigenetic phenotype variations. Functions of *endogenous RNA* ***isolated*** from total transcriptional output upon cell activation were investigated in vascular remodeling and tolerance phenomena. Structural codes *[3D-episcripts]* for epigenetic phenotyping were found escaping deciphering, so far. ***METHODS:*** Wissler et al, Protides Biol.Fluids **34**:517-536,1986; Materialwiss.Werkstofftech.**32**:984-1008,2001; Ann.N.Y.Acad.Sci.**961**:292-297,2002; **1022**:163-184,2004; **1137**:316-342,2008. ***RESULTS: Functional small ncRNA*** [<200n] were found upon cell activation by extrinsic environmental factors, including mitogenic, cell-mediated immune memory, metabolic [hypoxia] and [physical] shear stress reactions. They comprise conventional models for epigenetic remodeling variations directed rather to proteinaceous gene expression and regulation than genomic DNA sequence changes. Some edited, modified, *redox- and metalloregulated small hairpin* nc-shRNA bioaptamers are ***not complimentary*** to protein-coding transcripts, but feature 3D-episcripts fitting or misfitting to distinct protein conformers. Some address homologous helix-nucleating structural [proteomic] domains, termed **K**/**R**xxx**H** [**K**/**R**3**H**], i.e.-t/s/x**K**/**R**/q/n/hxxx**H**/y/n/q/e/d/r/kx$_{7\text{-}9}$h/xx$_{7\text{-}9}$h/xx$_{5\text{-}20}$**K**/**R**/q/n/e/h- with accessory canonical basic [R/K]$_n$, R/K-zipper, SR/K/RS, EF-hand and/or HxxxH/y/n/q segments, shared in several epigenetic regulator proteins entangled in growth, metabolic syndrome, vascularization, cancer epi- and genetic information indexing of the epigenome. ***CONCLUSIONS:*** Results suggest epigenetic [non-Mendelian] codes consisting of two different associated imprints: [1] Nucleic acid 3D-episcripts which some are not directly retranslatable to protein-coding transcripts. [2] Defined domains in epigenetic regulator protein and nucleic acid matrices as interaction address with [1] comprising all mutational, variant, polymorphism, infectious [viral] and Mendelian disease implications. This suggests tolerated mess-chaotic tumor vascularization associated with *bioaptamer disorders* in ncRNA-switched proteinaceous genetic and epigenetic processes.

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Linking Yeast Transcription Factor Structural Class and Detailed Binding Preferences with in vivo Regulatory Functions

Rachel Patton McCord[1], Cong Zhu[2], Trevor W. Siggers[2], Martha L. Bulyk[2].
[1]Harvard University, Cambridge, MA, USA, [2]Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.

Cellular responses to internal and external stimuli through changes in gene expression are in part controlled by the binding of regulatory transcription factors (TFs) to specific sequences of DNA. These TFs belong to a wide variety of DNA binding domain (DBD) structural classes. Sequence specificity is diverse between structural classes, but TFs within each class often have apparently redundant sequence preferences. To understand how cells use these regulators to coordinate networks of responses, it is essential to determine how regulatory function is shared and partitioned between factors of similar and different structures and DNA binding specificities. Are certain structural classes better suited for certain functions? Are detailed differences in binding preferences among apparently similar TFs relevant to *in vivo* function? Using genome-wide datasets, we have described trends in biological function and regulatory mechanisms within TF structural classes in the yeast *Saccharomyces cerevisiae*. These trends suggest general ways in which TF function may be distributed across structural classes according to the biophysical constraints dictated by each DBD structure. Such analyses do not show, however, how specific details of an individual TF's binding specificity might affect its biological function. New data from protein binding microarrays (PBMs) provide such detailed TF binding preference information at all possible 8 base-pair DNA sequences. By combining these PBM data with *in vivo* binding locations measured by chromatin immunoprecipitation (ChIP-chip) experiments, we can infer the functional importance of specific types of TF binding sites (ie low and high affinity sites). This and other analyses made possible by the high resolution PBM data, when combined with observed functional trends in structural classes, will demonstrate how the cell utilizes both general and specific biophysical TF properties to accomplish cellular functions.

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Dissecting the High Rate Constant for the Binding of a Ribotoxin to the Ribosome

Sanbo Qin, Huan-Xiang Zhou.
Florida State University, Tallahassee, FL, USA.

Restrictocin belongs to a family of site-specific ribonucleases that kill cells by inactivating the ribosome. The restrictocin-ribosome binding rate constant was observed to exceed 10^{10} $M^{-1}s^{-1}$ [1]. We have developed a transient-complex theory to model the binding rates of protein-protein and protein-RNA complexes [2, 3]. The theory predicts the rate constant as $k_a = k_{a0} \exp(-U_{el}^*/k_BT)$, where k_{a0} is the basal rate constant for reaching the transient complex, located at the outer boundary of the bound state, by random diffusion, and U_{el}^* is the average electrostatic interaction energy within the transient complex. Here we applied the transient-complex theory to dissect the high restrictocin-ribosome binding rate constant. We found that the binding rate of restrictocin to the isolated sarcin/ricin loop is electrostatically enhanced by ~300-fold, similar to results found in protein-protein and protein-RNA complexes [2, 3]. Ribosome provides an additional 5000-fold rate enhancement. Two mechanisms work together to provide the dramatic additional enhancement. First, with the ribosome the transient complex is formed with relative separations and orientations where local electrostatic interactions with sarcin/ricin loop are particularly favorable. Second, distant parts of the ribosome provides additional electrostatic attraction with the ribotoxin. Our results quantitatively rationalize the experimental results for salt dependences and mutational effects of the binding rates of restrictocin with the isolated sarcin/ricin loop and the ribosome.

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320-Pos Board B199

Insight into the Roles of the 140-149 Catalytic Loop and the Zinc-Binding Domain for HIV-1 Integrase Activity

Kevin Carayon, Li Na, Olivier Delelis, Francoise Simon, Jean-Francois Mouscadet, Jean-Francois Mouscadet, Jean-Claude Brochon, **Eric Deprez**.
CNRS, Cachan, France.

Compounds targeting HIV-1 integrase multimerization process represent an interesting approach with likely no cross-resistance with other compounds such as strand-transfer inhibitors (INSTI). To get deeper insight into the role of the zinc-binding domain into the multimerization process, we have studied the effect of a zinc ejector, DIBA-1(2,2′-dithiobisbenzamide-1), on integrase activity. In presence of Mg^{2+}, DIBA significantly decreases the Hill number characterizing integrase-DNA interaction, as measured by fluorescence anisotropy, with only modest effects on the overall affinity, suggesting that zinc ejection primarily perturbs protein-protein interactions and then the nature of the active oligomer. In presence of Mn^{2+}, we found that the DNA-binding of integrase was less cooperative and therefore the Mn^{2+}-dependent 3′-processing is not expected to be influenced by zinc ejection. Nevertheless, the Mn^{2+}-dependent disintegration activity performed by truncated integrase lacking the zinc-binding domain remains sensitive to DIBA-1. One residue, Cys56, was identified as playing an important role for DIBA efficiency in the Mn^{2+} context. In contrast, no effect of the C56 mutation was observed in presence of Mg^{2+}, suggesting that, in this context, DIBA primarily inhibits integrase by a zinc ejection mechanism. A catalytic mutant Q148H was also studied due to its key role in the resistance to Raltegravir, an INSTI currently used in clinic against viruses resistant to other antiretroviral compounds. An additional mutation, G140S, was found to be associated with Q148H in Raltegravir-resistant patients. We found that resistance was mainly due to Q148H as compared to G140S. However, Q148H alone ''freezes'' integrase into a catalytically inactive state. In contrast, the conformational transition from the inactive to the active form is rescued with the double mutation G140S/Q148H. Consequently, when combined together, G140S/Q148H lead to a highly resistant integrase with improved catalytic efficiency.

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Single Molecule Measurements Of The Role Of Tetramer Opening In LacI-mediated DNA Looping

Danielis Rutkauskas[1], **Francesco Vanzi**[1], Hongli Zhan[2], Kathleen S. Matthews[2], Francesco S. Pavone[1].
[1]European Laboratory for Nonlinear Spectroscopy (LENS), Sesto Fiorentino (FI), Italy, [2]Rice University, Houston, TX, USA.